

REMARKS

Claims 1-73 were pending in the instant application. Claims 1-53 and 59-70 were withdrawn from consideration. Claims 54-58 and 71-73 were under examination. By this amendment, claims 1-53, 59-68 and 71-73 are canceled without prejudice to Applicants' right to pursue the subject matter of the canceled claims in this or one or more related applications. Claims 54 and 57 are amended, and new claims 74-77 are added. In particular, claims 54 and 57 are amended to correct minor typographical errors and to recite that the mutein recombinant tissue protective cytokine (alternatively referred to herein as "EPO mutein") comprises the amino acid sequence of SEQ ID NO:10 with a non-conservative substitution of an amino acid residue at one or more of the indicated positions. Support for these amendments may be found in the specification as filed at, *e.g.*, page 39, line 33 to page 40, line 2; and on page 46, lines 25 to 26. Claim 57 is further amended to recite a method for "protecting, maintaining or enhancing the viability of a tissue" and to remove the term "or preventing." Support for this amendment may be found in the specification as filed at, *e.g.*, page 78, lines 24 to 30; and the Examples of Sections 6.6, 6.8, 6.9, 6.10, 6.11, 6.12, 6.13 and 6.18, starting at page 111. Claim 58 is amended to recite that the mammal has or is at risk for "injury from a surgical procedure, [or] injury from cardiopulmonary bypass." Support for this amendment may be found in the specification as filed at, *e.g.*, page 80, lines 10 to 15. Support for new claims 74-77 may be found in the specification as filed at, *e.g.*, page 46, line 25 to page 48, line 23; and page 49, lines 1 to 6. Thus, no new matter is added by the amendments to the claims. Upon entry of this amendment, claims 54-58 and 74-77 will be pending and under examination.

I. ELECTION/RESTRICTION REQUIREMENT

The Examiner's reconsideration and withdrawal of the finality of the restriction requirement for the species election to a single erythropoietin ("EPO") point mutation as previously applied to claims 54 and 57 is appreciated.

The Examiner has acknowledged Applicants' traverse of the withdrawal of claims 69 and 70. Nonetheless, the Examiner has deemed their withdrawal proper as being drawn to a non-elected species. The Examiner's explanation for rendering the species election proper is

that Applicants elected, without traverse, "retinal ischemia" as the species of injury, and that each of the listed injury types represents a patentably distinct invention and would require a separate search of the art that would be burdensome to the Examiner. Applicants respectfully disagree. The recitation in claim 69 of administering a mutein recombinant tissue protective cytokine (referred to alternatively herein as an "EPO mutein") "prior to a surgical procedure" falls within the scope of claim 57 (reciting a "method for protecting against or preventing a tissue injury") and the species election of "retinal ischemia," and as such should be examined. Therefore, Applicants respectfully request reconsideration and withdrawal of the species election. Attorneys for Applicants retain the right to petition from the restriction requirement under 37 C.F.R. § 1.144.

Finally, Applicants respectfully request that upon allowance of a claim(s) drawn to the elected species, withdrawn claims that depend from or otherwise include all the limitations of the allowable claim should be rejoined in accordance with the provisions of M.P.E.P. § 821.04. Upon allowance of a generic claim, claims drawn to unelected species that include all the limitations of the generic claim should be allowable.

II. WITHDRAWN OBJECTIONS AND/OR REJECTIONS

Applicants acknowledge with appreciation Examiner's withdrawal of all claim objections and rejections not reiterated in the present Office Action. Applicants acknowledge with appreciation Examiner's withdrawal of the rejection of claims 54-58 under 35 U.S.C. § 102(b) over *Campana et al.*

III. THE REJECTIONS OF CLAIMS 54-58 FOR OBVIOUSNESS-TYPE DOUBLE PATENTING SHOULD BE WITHDRAWN

In the Office Action, the rejections of claims 54-58, first made in the Office Action mailed February 23, 2007, under the judicially created doctrine of obviousness-type double patenting have been maintained.

Claim 57 was rejected for alleged obviousness-type double patenting over claims 1-10 of U.S. Patent No. 6,531,121 B2 (the "'121 patent"). Without admitting to the merits of the rejection, Applicants respectfully request the withdrawal of the double patenting rejection over the '121 patent in view of the amendments to instant claim 57. The method of amended

claim 57 is not an obvious variation of the methods claimed in the '121 patent, because the latter do not recite the limitations of claim 57, *i.e.*, EPO muteins comprising a non-conservative substitution of an amino acid residue at one or more of the amino acid positions 11 to 15, 44 to 51, 100 to 108 or 146 to 151. Moreover, the Examiner's reliance on the specification of the '121 patent is improper because the specification of a patent underlying a double patenting rejection cannot be used as a reference under 35 U.S.C. 103. *In re Vogel*, 422 F.2d 438, at 925 (CCPA 1970). Accordingly, Applicants respectfully request the withdrawal of the rejection of claim 77 for double patenting over the '121 patent.

Claims 54-58 were provisionally rejected for alleged obviousness-type double patenting over claims 35 and 37-38 of copending Application No. 10/188,905. Claims 57 and 58 were provisionally rejected for alleged obviousness-type double patenting over claims 1 and 15 of copending Application No. 09/716,960 (now U.S. Patent No. 7,410,941, issued August 12, 2008). Claims 54-56 were provisionally rejected for alleged obviousness-type double patenting as being unpatentable over claims 2 and 5 of copending Application No. 10/351,640. Claim 57 was provisionally rejected for alleged obviousness-type double patenting as being unpatentable over claim 1 of copending Application No. 10/185,841 and over claims 1 and 4 of copending Application No. 10/573,905 (which will issue as U.S. Patent No. 7,645,733 on January 12, 2010). Applicants request that these provisional double patenting rejections continue to be held in abeyance until indication of allowable subject matter in the present application.

IV. THE REJECTIONS UNDER 35 U.S.C. § 112, FIRST PARAGRAPH, FOR LACK OF ENABLEMENT, SHOULD BE WITHDRAWN

Claims 54-58 and 71-73 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the enablement requirement. Applicants acknowledge with appreciation the Examiner's conclusion at page 6, ¶ 12 of the Office Action that the specification is enabling for

- (i) a method of protecting, maintaining or enhancing the viability of an erythropoietin-sensitive cell, tissue or organ isolated from a mammalian body; or

- (ii) a method for protecting from tissue injury, or restoring or rejuvenating tissue function in a mammal wherein the tissue is responsive to EPO; comprising exposing said cell, tissue or organ, in vivo or in vitro to a pharmaceutical composition comprising a mutein tissue protective cytokine comprising SEQ ID NO:10, wherein the mutein consists of S100E, R150E, R103E, K45D or K45D/S100E, and has a reduced level of in vivo erythropoietic activity.

However, the Examiner contends that the specification does not reasonably provide enablement for the method of comprising exposing said cell, tissue or organ in vivo or in vitro, to a pharmaceutical composition comprising any mutein recombinant tissue protective cytokine comprising SEQ ID NO:10, with a substitution of any amino acid residue at one or more positions of SEQ ID NOs: 1, 2, 3 or 4. The Examiner further asserts that the specification is not enabled for the prevention of a tissue injury or restoring or rejuvenating tissue in a mammal comprising administering any mutein tissue protective cytokine.

Without agreeing to the merits of the rejection, and solely to expedite allowance of the claims, Applicants have canceled claims 72 and 73, have amended claims 54 and 57 to recite that the EPO muteins comprise non-conservative mutations at the recited amino acid positions, and have additionally amended claim 57 to remove the terms “preventing” and restoring or rejuvenating “tissue” in a mammal. Applicants believe the rejections for lack of enablement should be withdrawn for the following reasons.

The Legal Standard

The test for enablement is whether one reasonably skilled in the pertinent art could make or use the invention, without undue experimentation, from the disclosure in the patent specification coupled with information known in the art at the time the patent application was filed. *U.S. v. Electronics Inc.*, 857 F.2d 778, 8 USPQ2d 1217 (Fed. Cir. 1988).

Undue experimentation is experimentation that would require a level of ingenuity beyond what is expected from one of ordinary skill in the field. *Fields v. Conover*, 170 USPQ 276, 279 (CCPA 1971). The factors that can be considered in determining whether an amount of experimentation is undue have been listed in *In re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988). Among these factors are: the amount of effort

involved, the guidance provided by the specification, the presence of working examples, the amount of pertinent literature and the level of skill in the art. The test for undue experimentation is not merely quantitative, since a considerable amount of experimentation is permissible, so long as it is merely routine. *Id.*

Further, while the predictability of the art can be considered in determining whether an amount of experimentation is undue, mere unpredictability of the result of an experiment is not a consideration. Indeed, the Court of Custom and Patent Appeals specifically cautioned that the unpredictability of the result of an experiment is not a basis to conclude that the amount of experimentation is undue:

[If to fulfill the requirements of 112, first paragraph, an applicant's] disclosure must provide guidance which will enable one skilled in the art to determine, with reasonable certainty before performing the reaction whether the claimed product will be obtained, ... then all "experimentation" is "undue" since the term "experimentation" implies that the success of the particular activity is uncertain. Such a proposition is contrary to the basic policy of the Patent Act.

In re Angstadt, 190 USPQ 214 (CCPA 1976), at 219.

Thus, all that is required is a reasonable amount of guidance with respect to the direction of the experimentation; reasonable certainty with regard to the outcome of the experimentation is not required.

In a more recent decision of the Board of Patent Appeals and Interferences ("Board"), the Board applied the principles of *Angstadt* to a biotechnological context. *In re Neuberger & Rabbitts*, 2002 WL 33952578 (B.P.A.I. 2002). The rejected claims in *Neuberger* were directed to chimeric antibodies with (i) a certain antigen binding activity; and (ii) a certain biological activity. The Examiner in *Neuberger* had rejected these claims based on lack of predictability. The Board reversed the Examiner's decision because "a requirement for certainty would be incompatible with any amount of experimentation and therefore incompatible with the standard of enablement." *Id.* at 3.

The Claimed EPO Muteins for Tissue Protection Are Enabled

By amending claims 54 and 57 to recite that substitution(s) in amino acids 11-15 (VLQRY), amino acids 44-51 (TKVNIFYAW), 100-108 (SGLRSLTTL) or 146-151 (SNFLRG) are non-conservative, the number of possible EPO sequences encompassed by the claims is reduced. Because of their non-conservative character, these amino acid substitutions are likely to reduce the biological activity of the protein regions where the substitutions are located. The regions specified in the claims are required for erythropoietic activity of EPO and, thus, a non-conservative amino acid substitution is likely to reduce the erythropoietic activity of EPO.

Support for these amendments may be found in the specification at, e.g., page 39, line 33 to page 40, line 2; and on page 46, lines 25 and 26. The meaning of the term “non-conservative” would have been clear to one of skill in the art as of the filing date of the instant application. Non-conservative amino acid substitutions are amino acid substitutions wherein the original amino acid and the new amino acid fall within different families of amino acids listed at page 39, lines 26 to 28 and/or page 46, line 28 to page 47, line 5. Thus, non-conservative substitutions comprise replacing an amino acid in a recited region with another that is dissimilar in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or amphipathicity (see, e.g., page 39, lines 26 to 28). Examples of such substitutions within the regions defined by SEQ ID NOs:1-4 are among those provided at page 47, line 24 and lines 30 to 31; and page 48, lines 4 to 5 and line 13. Others are among those described at page 6, line 25 to page 8, line 25; and page 33, line 11 to page 34, line 25.

In view of the teachings of the specification of the instant application, the skilled artisan would understand that a non-conservative amino acid substitution(s) within EPO amino acids 11-15 (VLQRY), amino acids 44-51 (TKVNIFYAW), 100-108 (SGLRSLTTL) or 146-151 (SNFLRG) is likely to impair its interaction with the erythropoietic EPO receptor and reduce its erythropoietic activity without interfering with tissue protective functionality (see page 39, line 22 to page 40, line 6). Indeed, as shown in the working examples of the specification, EPO muteins with the non-conservative mutations S100E, R150E, R103E, K45D and K45D/S100E (uncharged polar to acidic, basic to acidic, basic to acidic, basic to

acidic, and basic to acidic/uncharged polar to acidic, respectively) have reduced erythropoietic activity but possess tissue protective activity. See, e.g., Amendment filed June 30, 2008, a copy of which is attached as Exhibit A, at pages 31-34. Thus, the specification in view of the state of the art provides sufficient guidance for the skilled artisan to identify the EPO muteins encompassed by the claims.

Once the EPO muteins encompassed by the claims are identified, one of skill in the art reading the specification as of the instant filing date would have been able to make and test the EPO muteins for tissue protective functionality without undue experimentation. See, Amendment filed on June 30, 2008 at pages 30-35. No undue experimentation would be required to do so because a considerable amount of experimentation is permissible, so long as it is merely routine. See, e.g., *In re Wands*, 858 F.2d 731 (Fed. Cir. 1988). In *Wands*, antibodies that were required to perform the claimed immunoassays were held to be enabled even though only a *small percentage* of hybridomas were proved to fall within the claims. *Id.* at 739. In *Wands* routine assays could be used to identify the suitable hybridomas. Similarly, in the present situation, routine assays can be used to confirm those EPO muteins that have reduced erythropoietic activity and tissue protective functionality, for example, as exemplified in the working examples of the specification for the EPO muteins S100E, R150E, R103E, K45D and K45D/S100E.

Despite the instant specification's teaching of how to identify, make, and use the EPO muteins provided in the claims, and the experimental data provided therein with respect to the five EPO muteins, S100E, R150E, R103E, K45D and K45D/S100E, the Examiner contends that the specification has not provided sufficient guidance about specific muteins demonstrating the claimed properties (Office Action, page 10). The Examiner states that "in all likelihood" sequences with mutations in the aforementioned four regions will not result in the desired activity. In support of this contention, the Examiner provides Gantier *et al.* (U.S. Patent Application Publication No. 20080194477A1, dated August 14, 2008; "Gantier") as an example of a publication that provides EPOs with point mutations that have increased bioavailability and resistance to proteolysis and have the same therapeutic properties of the unmodified cytokine involving erythropoietic activity. The Examiner states that Gantier

provides many examples of EPO muteins that do not have a reduction in erythropoietic activity. Applicants respectfully disagree.

Gantier did not make EPO muteins that do not have a reduction in erythropoietic activity, and therefore Gantier does not support the Examiner's contention that the presently claimed invention is not enabled. Gantier, a post-filing reference, provides a generic roadmap for making cytokine mutants (including EPOs) that protect against proteolysis and which may retain their biological function. Contrary to the Examiner's assertion, there is no evidence in Gantier Fig. 12L or elsewhere that K45Q or other EPO muteins provided therein protect against proteolysis without reducing erythropoietic activity. Fig. 12L only provides EPO mutations predicted to improve stability (see, e.g., Gantier, ¶ 0058). And the Table at page 103 of Gantier provides exemplary biological functions that may be maintained (were one to test them) when such mutations are made, and the listed biological functions are not limited to EPO's erythropoietic activity. Thus, it is possible that the biological activity retained by some or all of Gantier's EPO mutants is not erythropoietic activity. Thus, absent evidence to the contrary, Applicants respectfully assert that there is no reason to conclude from Gantier that substitutions among amino acids 11-15, 44-51, 100-108 or 146-151 of EPO preserve erythropoietic activity and, thus, serve as evidence for the nonenablement for the presently claimed invention.

The Examiner also contends that the specification fails to provide guidance on the making and using of specific muteins that will satisfy the claimed requirement of tissue protection as broadly claimed. In particular, the Examiner accepts Applicants' argument that because the tissue protective receptor complex is present in a wide variety of tissues, EPO muteins with the claimed activity will exhibit protection in a wide range of responsive cells and tissues in different injury states. However, the Examiner appears to object to the fact that the claims read broadly on a "huge number of muteins," entailing undue experimentation in recognizing the right mutein having the claimed characteristics. In response, and as explained in the foregoing paragraphs, Applicants respectfully assert that no undue experimentation is required to practice the invention as set forth in the claims as amended. In particular, by limiting the number of EPO muteins encompassed by the claims, and in view of the teachings of the specification, it would have required no more than routine

experimentation to make and use EPO muteins in accordance with the claimed methods of tissue protection. Furthermore, without agreeing to the merits of the rejection, and solely to expedite allowance of the claims, the terms “prevention” and restoring or rejuvenating “tissue” have been removed from claim 57, and accordingly, the Examiner’s rejection of the claims for nonenablement over these two issues (see Office Action, pages 13 to 14, end of ¶ 19 to ¶ 20) is moot.

In conclusion, in view of the foregoing comments and amendments, Applicants respectfully assert that there is no undue experimentation to practice the presently claimed invention, and accordingly request the withdrawal of the rejection for lack of enablement under 35 U.S.C. § 112, first paragraph.

V. THE REJECTIONS UNDER 35 U.S.C. § 112, FIRST PARAGRAPH, FOR INSUFFICIENT WRITTEN DESCRIPTION, SHOULD BE WITHDRAWN

Claims 54-58 and 71-73 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement. The Examiner contends that the claims as (previously) amended fail to recite a representative number of EPO mutein species and hence the specification does not provide adequate written description of methods using an entire genus of EPO muteins capable of tissue protective activity along with reduced in vivo erythropoietic activity as broadly claimed. While the Examiner agrees that the specification provides sufficient written description with regard to EPO muteins S100E, R150E, R103E, K45D or K45D/S100E and methods for their use, the Examiner contends that the specification does not reasonably provide sufficient written description of methods using the entire claimed genus of EPO muteins.

Without agreeing to the merits of the rejection, and solely to expedite allowance of the claims, Applicants have canceled claims 72 and 73, have amended claims 54 and 57 to recite that the EPO muteins comprise non-conservative mutations at the specified amino acid positions. In view of these amendments, Applicants believe the rejections for insufficient written description are moot for the following reasons.

The Legal Standard

The test for the sufficiency of written description is whether the disclosure of the application ‘reasonably conveys to the artisan that the inventor had possession’ of the claimed subject matter. *In re Kaslow*, 707 F.2d 1366, 1375, 217 U.S.P.Q. (BNA) 1089, 1096 (Fed. Cir. 1983); accord *Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555, 1563; *see also, Ralston Purina Co. v. Far-Mar-Co, Inc.*, 772 F.2d 1570, 1575, 227 U.S.P.Q. (BNA) 177, 179 (Fed. Cir. 1985). The Court of Appeals for the Federal Circuit has repeatedly considered the written description requirement and consistently found that exacting detail is not necessary to meet the requirement:

If a person of ordinary skill in the art would have understood the inventor to have been in possession of the claimed invention at the time of filing, even if [not] every nuance of the claims is explicitly described in the specification, the adequate written description requirement is met. *In re Alton*, 76 F.3d 1168, 37 USPQ2d 1578 (Fed. Cir. 1996).

A claimed genus may be described by disclosing: “(1) a representative number of species in that genus; or (2) its ‘relevant identifying characteristics,’ such as ‘complete or partial structure, other physical and/or chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics.’” *In re Alonso*, 545 F.3d 1015, 1019, 88 USPQ2d 1849, at 1852 (Fed. Cir. 2008).

The Claimed EPO Muteins for Tissue Protection Are Sufficiently Described

Claims 54 and 57 are amended to recite that substitution(s) in amino acids 11-15 (VLQRY), amino acids 44-51 (TKVNFYAW), 100-108 (SGLRSLTTL) or 146-151 (SNFLRG) are non-conservative. These structural features of these EPO muteins are correlated with their function. In particular, because of their non-conservative character, the amino acid substitutions are likely to reduce the biological activity of the regions where the substitutions are located. The regions specified in the claims are required for erythropoietic activity of EPO and, thus, a non-conservative amino acid substitution is likely to reduce the erythropoietic activity of EPO.

Specific EPO muteins that may be used in the presently claimed invention are described throughout the specification. Non-conservative amino acid substitutions are amino acid substitutions wherein the original amino acid and the new amino acid fall within different families of amino acids listed at page 39, lines 26 to 28 and/or page 46, line 28 to page 47, line 5. Thus, non-conservative substitutions comprise replacing an amino acid in a recited region with another that is dissimilar in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or amphipathicity (see, *e.g.*, page 39, lines 26 to 28). Examples of such substitutions within the regions defined by SEQ ID NOs:1-4 are among those provided at page 47, line 24 and lines 30 to 31; and page 48, lines 4 to 5 and line 13. Others are among those described at page 6, line 25 to page 8, line 25; and page 33, line 11 to page 34, line 25. Thus, contrary to the Examiner's assertion at page 16 of the Office Action that there is no means by which the artisan would know whether a given EPO mutein was a member of the claimed genus, Applicants believe that the specification provides a clear disclosure of the EPO muteins that fall within the claimed genus.

The structure of the EPO muteins recited in the claims (one or more non-conservative mutations in the four regions required for binding to the erythropoietic EPO receptor, *i.e.*, at amino acid positions 11-15, 44-51, 100-108, or 146-151) is correlated with their function – reduced erythropoietic activity (as a result of affecting the ability to bind to the erythropoietic EPO receptor) yet maintained tissue protective functionality – such that there is sufficient evidence that Applicants were in possession of the claimed invention as of the instant filing date. Indeed, as explained in the foregoing discussion with regard to the enablement of claimed invention, the experimental data provided in the specification on five EPO muteins, S100E, R150E, R103E, K45D and K45D/S100E, makes this manifest.

In conclusion, the specification of the instant application provides sufficient written description for the claimed invention by virtue of (i) describing numerous EPO muteins that fall within the scope of the claims, including numerous working examples, and a (ii) disclosed correlation between structure and function, such that a skilled artisan would recognize that Applicants were in possession of the claimed invention at the time of filing. Thus, Applicants respectfully request withdrawal of the rejection under 35 U.S.C. § 112, first paragraph, for lack of written description.

VI. THE REJECTIONS UNDER 35 U.S.C. § 102 SHOULD BE WITHDRAWN

Claims 54-58 and 71-73 are rejected under 35 U.S.C. § 102(e), as allegedly being anticipated by International Application Publication No. WO 02/053580 to Brines *et al.*, filed December 28, 2001 ("Brines"). The Examiner asserts that Brines teaches the specific EPO muteins recited in the (previously) amended claims, and teaches the structural and functional limitations of the claims.

Without agreeing to the merits of the rejection, and solely to expedite allowance of the claims, Applicants have canceled claims 71-73 and have amended claims 54-58 to include the limitation that the mutations are non-conservative mutations. In view of this amendment, Applicants believe this rejection is moot. In particular, Applicants submit that Brines does not anticipate the claims as amended because Brines does not teach all claim limitations. In particular Brines fails to disclose non-conservative amino acid substitutions. Therefore, Brines does not anticipate the specific EPO mutein species of the amended claims. *Cf. Impax Laboratories, Inc. v. Aventis Pharmaceuticals Inc.*, 468 F.3d 1366 (Fed. Cir. 2006) (holding that a prior patent containing a formula including hundreds of compounds does not anticipate the specific compound of the claims, and the specific compound was not defined in the prior patent).

Accordingly, Applicants assert that amended claims 54-58 are not anticipated by Brines and respectfully request withdrawal of the rejection for anticipation under 35 U.S.C. § 102(e).

CONCLUSION

Entry of the foregoing amendments and remarks into the record of the above-identified application is respectfully requested.

Respectfully submitted,

Date: January 11, 2010

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Enclosures

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EXHIBIT A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Nielsen *et al.*

Confirmation No.: 5726

Serial No.: 10/612,665

Art Unit: 1649

Filed: July 1, 2003

Examiner: Dutt, Aditi

For: RECOMBINANT TISSUE PROTECTIVE
CYTOKINES AND ENCODING NUCLEIC
ACIDS THEREOF FOR THE PROTECTION,
RESTORATION AND ENHANCEMENT OF
RESPONSIVE CELLS, TISSUES AND
ORGANS

Attorney Docket No: 10165-022-999

AMENDMENT UNDER 37 C.F.R. § 1.111

MAIL STOP AMENDMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In response to the non-final Office Action dated December 31, 2007 ("Office Action") and pursuant to the Rules of Practice, please enter the following amendments and consider the following remarks. Enclosed herewith are: (i) a Petition for Extension of Time for three (3) months from March 31, 2008 to and including June 30, 2008 with a provision authorizing payment of the required fee (in duplicate); (ii) an Amendment Fee Transmittal Sheet (in duplicate); (iii) Transmittal of Substitute Sequence Listing (in duplicate); (iv) Substitute Sequence Listing in paper and computer-readable format; (v) Exhibit A: filing receipt for Application Serial No. 10/188,905; (vi) Exhibit B: copies of Webster's Ninth New Collegiate Dictionary, Springfield, MA: Merriam-Webster, Inc., 1986, pp. 994 and 1005, entries for "rejuvenate" and "restore," respectively; (vii) Appendix A: Replacement table at pages 69-71 of specification; (viii) a Declaration of Michael L. Brines, M.D., Ph.D. ("Brines Declaration"), with Appendices A-E; and (ix) Supplemental Information Disclosure Statement (in duplicate) with revised form PTO 1449 entitled "List of References Cited By Applicant," accompanied by copies of references A73-A81, B09-B43, and C123-C239.

Applicants respectfully request that the amendments and remarks made herein be entered into the record of the instant application. Please charge all required fees to Deposit Account No. 50-3013.

Amendments to the Specification begin on page 3 of this paper.

Amendments to the Claims begin on page 10 of this paper.

Remarks begin on page 24 of this paper.

AMENDMENTS TO THE SPECIFICATION:

Please replace the paragraph at p. 9, l. 27 to p. 10, l. 5 with the following amended paragraph:

Moreover, such aforementioned recombinant tissue protective cytokines may be further modified by having a chemical modification of one or more amino acids, such as described in the following co-pending applications: PCT application serial no. PCT/US01/49479, filed December 28, 2001, U.S. Patent Application Serial No. 09/753,132 filed December 29, 2000, and U.S. Patent Application ~~Attorney Docket No. KW00-009C02- US 10/188,905~~ filed July 3, 2002, each of these applications is incorporated herein by reference in their entirety. These further chemical modifications may be used to enhance the tissue protective activities of the recombinant tissue protective cytokines or suppress any effects the recombinant tissue protective cytokines may have on bone marrow. In a further embodiment, the additional chemical modification is provided to restore solubility of the molecule that may be reduced as a result of the aforementioned genetic modification, such as chemically adding a positive or negative charge to the molecule if a charged amino acid residue is changed to an uncharged residue.

Please replace the paragraph at p. 10, ll. 7-16, with the following amended paragraph:

By way of non-limiting examples, recombinant tissue protective cytokines of the invention include human erythropoietin mutein S100E (~~SEQ ID NO:5~~) (SEQ ID NO:62), human erythropoietin mutein K45D (~~SEQ ID NO:6~~) (SEQ ID NO:44), and any of the nonerythropoietic yet cellular protective recombinant tissue protective cytokines or those able to benefit a responsive cell, tissue or organ, that are described in Elliott *et al.*, 1997, Blood 89:493-502; Boissel *et al.*, Journal of Biological Chemistry, vol. 268, No. 21, pp. 15983-15993 (1993); Wen *et al.*, Journal of Biological Chemistry, vol. 269, No. 36, pp. 22839-22846 (1994); and Syed *et al.*, Nature, vol. 395, pp. 511-516 (1998), which are incorporated herein by reference in their entirety. The present invention is directed to methods for the use of any of the aforementioned recombinant tissue protective cytokines for the protection, restoration, and enhancement of responsive cells, tissues, and organs.

Please replace the paragraph at p. 16, ll. 9-16, with the following amended paragraph.

According to one aspect of the invention, there is provided an isolated nucleic acid molecule that comprises a nucleotide sequence which encodes a polypeptide comprising the recombinant tissue protective cytokine as described herein above. In one embodiment, the isolated nucleic acid molecule comprises the nucleotide sequence of nucleotide residues 5461 through 6041 of the ~~vector construct~~ vector construct of SEQ ID NO: 208, nucleotide residues 5461 through 6041 of SEQ ID NO: 209, nucleotide residues 5461 through 6041 of SEQ ID NO: 210, nucleotide residues 5461 through 6041 of SEQ ID NO: 211, or nucleotide residues 5461 through 6041 of ~~SEQ ID NO: 212~~ SEQ ID NO: 5.

Please replace the paragraph at p. 20, ll. 3-17 with the following amended paragraph:

The invention further provides for the use of a recombinant tissue protective cytokine as described herein above, that lacks at least one erythropoietic activity selected from the group consisting of increasing hematocrit, vasoactive action (vasoconstriction/vasodilatation), hyperactivating platelets, pro-coagulant activity and increasing production of thrombocytes, for the preparation of a pharmaceutical composition for the protection against and prevention of a tissue injury as well as the restoration of and rejuvenation of tissue and tissue function in a mammal. In one embodiment, the injury is caused by a seizure disorder, multiple sclerosis, stroke, hypotension, cardiac arrest, ischemia, myocardial infarction, inflammation, age-related loss of cognitive function, radiation damage, cerebral palsy, neurodegenerative disease, Alzheimer's disease, Parkinson's disease, Leigh's disease, AIDS dementia, memory loss, amyotrophic lateral sclerosis, alcoholism, mood disorder, anxiety disorder, attention deficit disorder, hyperactivity, autism, ~~Creutzfeldt-Jakob~~ Creutzfeldt-Jakob disease, brain or spinal cord trauma or ischemia, heart-lung bypass, chronic heart failure, macular degeneration, diabetic neuropathy, diabetic retinopathy, glaucoma, retinal ischemia, or retinal trauma.

Please replace the paragraph at p. 39, l. 8 to p. 40, l. 14 with the following amended paragraph:

Recombinant tissue protective cytokines of the invention include erythropoietin muteins, that maintain partial or full erythropoietic activity. Erythropoietin is a glycoprotein

hormone which in humans has a molecular weight of about 34 kDa. The mature protein comprises 165 amino acids (SEQ ID NO:10), and the glycosyl residues comprise about 40% of the weight of the molecule. The forms of recombinant tissue protective cytokine useful in the practice of the present invention encompass at least a single amino acid change in naturally-occurring, synthetic and recombinant forms of the following human and other mammalian erythropoietin-related molecules: erythropoietin, asialoerythropoietin, deglycosylated erythropoietin, erythropoietin analogs, erythropoietin mimetics, erythropoietin fragments, hybrid erythropoietin molecules, erythropoietin receptor-binding molecules, erythropoietin agonists, renal erythropoietin, brain erythropoietin, oligomers and multimers thereof, and congeners thereof. Such equivalent recombinant tissue protective cytokines include mutant erythropoietins, which may contain substitutions, deletions, including internal deletions, additions, including additions yielding fusion proteins, or conservative substitutions of amino acid residues within and/or adjacent to the amino acid sequence, but that result in a "silent" change, in that the change produces a functionally equivalent erythropoietin mutein or recombinant tissue protective cytokine. In a preferred embodiment, the recombinant tissue protective cytokine is nonerythropoietic, i.e. lacking or exhibiting diminished erythropoietic activity. Conservative amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Alternatively, non-conservative amino acid changes, and larger insertions and deletions may be used to create functionally altered recombinant tissue protective cytokines. Such mutants can be used to alter erythropoietin properties in desirable ways. For example, in one embodiment, an erythropoietin useful for the practice of the invention can be a recombinant tissue protective cytokine altered in one or more amino acids within the four functional domains of erythropoietin which affect receptor binding: VLQRY (SEQ ID NO:1) and/or TKVNIFYAW (SEQ ID NO:2) and/or SGLRSLTTL (SEQ ID NO:3) and/or SNFLRG (SEQ ID NO:4). In another embodiment, erythropoietins containing mutations in the surrounding areas of the molecule which affect the kinetics or

receptor-binding properties of the molecule can be used. Determining which alterations, or which positions in the domains will effect binding can be accomplished using standard methods. For example, the domains may be altered by pair-wise alanine mutations (alanine scanning mutagenesis) followed by measurement of binding kinetics of mutants to examine the effect on binding to a receptor (Bernat *et al.*, 2003, PNAS 100:952-957; Wells *et al.*, 1989, Science 244:1081-1085).

Please replace the paragraph at p. 42, ll. 1-23 with the following amended paragraph:

Furthermore, derivative recombinant tissue protective cytokine molecules desirable for the uses described herein may be generated by guanidination, amidination, carbamylation (carbamoylation), trinitrophenylation, acylation such as acetylation or succinylation, nitration, or modification of arginine, aspartic acid, glutamic acid, lysine, tyrosine, tryptophan, or cysteine residues or carboxyl groups, among other procedures, such as limited proteolysis, removal of amino groups, and/or mutational substitution of arginine, lysine, tyrosine, tryptophan, or cysteine residues by molecular biological techniques to produce erythropoietin muteins or recombinant tissue protective cytokines which maintain an adequate level of activities for specific organs and tissues but not for others, such as erythrocytes (*e.g.*, Satake *et al.*; 1990, *Biochim. Biophys. Acta* 1038:125-9; incorporated herein by reference in its entirety[~~]]~~), in which *in vivo* biological activity was determined by the polycythemic mouse bioassay). One non-limiting example as described hereinbelow is the modification of erythropoietin arginine residues by reaction with a glyoxal such as phenylglyoxal (according to the protocol of Takahashi, 1977, *J. Biochem.* 81:395-402). As will be seen below, such a recombinant tissue protective cytokine molecule fully retains the neurotrophic effect of erythropoietin. Such recombinant tissue protective cytokine molecules are fully embraced for the various uses and compositions described herein. In addition, these chemical modifications may be further used to enhance the protective effects of the recombinant tissue protective cytokines or neutralize any changes in the charge of the molecule resulting from the amino acid mutation of the native erythropoietin. Such modifications are described in co-pending applications:[~~]]~~] serial no. PCT/US01/49479, filed December 28, 2001; serial no. 09/753,132, filed December 29, 2000 and ~~Attorney's Docket~~

No. K/W00-009C02-US serial no. 10/188,905, filed July 3, 2002, all of which are incorporated herein in their entireties.

Please replace the paragraph at p. 47, ll. 11-22 with the following amended paragraph:

Further to the above-mentioned erythropoietin modifications useful herein, the following discussion expands on the various recombinant tissue protective cytokines of the invention. As described in Elliott *et al.*, Boissel *et al.*, and Wen *et al.*, mentioned above, the following erythropoietin muteins are useful for the purposes described herein, and may be provided in a pharmaceutical composition for the methods herein. In the mutein nomenclature used throughout herein, the changed amino acid is depicted with the native amino acid's one-letter code first, followed by its position in the erythropoietin molecule, followed by the replacement amino acid one-letter code. For example, "human erythropoietin S100E" or "recombinant ~~tissue protective~~ tissue protective cytokine S100E" refers to a human erythropoietin molecule in which, at amino acid position 100 of the mature erythropoietin, a serine has been changed to glutamic acid. Such muteins useful for the practice of the present invention include but are not limited to human erythropoietin with at least one of the following amino acid changes:

Please replace the paragraph at p. 68, ll. 6-25 with the following amended paragraph:

Various neuropsychologic disorders which are believed to originate from excitable tissue damage are treatable by the instant methods. Chronic disorders in which neuronal damage is involved and for which treatment by the present invention is provided include disorders relating to the central nervous system and/or peripheral nervous system including age-related loss of cognitive function and senile dementia, chronic seizure disorders, Alzheimer's disease, Parkinson's disease, dementia, memory loss, amyotrophic lateral sclerosis, multiple sclerosis, tuberous sclerosis, Wilson's Disease cerebral and progressive supranuclear palsy, Guam disease, Lewy body dementia, prion diseases, such as spongiform encephalopathies, *e.g.*, Creutzfeldt-Jakob disease, Huntington's disease, myotonic dystrophy, ~~Friedrich's~~ Friedrich's ataxia and other ataxias, as well as Gilles de la Tourette's syndrome, seizure disorders such as epilepsy and chronic seizure disorder, stroke, brain or spinal cord trauma, AIDS dementia, alcoholism, autism, retinal ischemia, glaucoma, autonomic function

disorders such as hypertension and sleep disorders, and neuropsychiatric disorders that include, but are not limited to, schizophrenia, schizoaffective disorder, attention deficit disorder hyperactivity, dysthymic disorder, major depressive disorder, mania, obsessive-compulsive disorder, psychoactive substance use disorders, anxiety, panic disorder, as well as unipolar and bipolar affective disorders. Additional neuropsychiatric and neurodegenerative disorders include, for example, those listed in the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (DSM), the most current version, IV, of which is incorporated herein by reference in its entirety.

Please replace the paragraph at p. 89, ll. 14-20 with the following amended paragraph:

The PCR product was cloned between the Xho I and Xba I sites in pCiNeo mammalian expression vector (Promega). The clones were sequenced and the sequence was verified to match the sequence in NM_000799 with the exception of a single base. Base 418 in the coding sequence (starting the numbering from the ATG) was C instead of G, changing amino acid 140 in the full length EPO sequence (*i.e.*, SEQ ID NO:6) starting from the first methionine from Arg to Gly. This is, however, normal sequence variation from the original sequence and present in most forms of erythropoietin.

Please replace the three paragraphs at p. 90, ll. 4-10 with the following amended three paragraphs:

This cDNA codes for the full length amino acid sequence of erythropoietin, which is below

MGVHECPAWLWLLSLSLPLGLPVLGAPPRICDSRVLERYLLEAKEAENIT
TGCAEHCSLNENITVPDTKVNIFYAWKRMEVGQQAVEVWQGLALLSEAVLRGQALL
VNSSQPWEPLQLHVDKAVSGLRSLTLLRALGAQKEAISPPDAASAAPLRTITADTRF
KLFRVYSNFLRGKCLKLYTGEACRTGDR (~~SEQ ID NO: 10~~) (SEQ ID NO:6).

The first 27 amino acid residues of ~~SEQ ID NO: 10~~ SEQ ID NO:6 comprise a leader sequence.

Please replace the paragraph at p. 101, ll. 19-25 with the following amended paragraph:

The following are examples of constructs that were made: human EPO(hEPO)-6xHisTag-pCiNeo sequence (SEQ ID NO: 208); hEPO6xHisTag-A30N/H32T-pCiNeo (SEQ ID NO: 209); hEPO-6xHisTag-K45D-pCiNeo sequence (SEQ ID NO: 210); hEPO-6xHisTag-S100E-pCiNeo sequence (SEQ ID NO: 211); and hEPO-6xHisTag-K45D/S100E-pCiNeo sequence (~~SEQ ID NO: 212~~) (SEQ ID NO: 5). The pCI-neo mammalian expression vector carries the human cytomegalovirus (CMV) immediate-early enhancer/promoter region to promote constitutive expression of cloned DNA inserts in mammalian cells.

Please replace the table at pp. 69-71 with the replacement table submitted herewith as Appendix A. The table is amended to replace the term "Guillian Barre" with the corrected term "Guillain Barre" (see specification as filed at p. 71, l. 13).

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of the claims:

1. (Withdrawn) A mutein recombinant tissue protective cytokine lacking at least one activity selected from the group consisting of increasing hematocrit, vasoactive action, hyperactivating platelets, pro-coagulant activities and increasing production of thrombocytes, the cytokine comprising at least one responsive cellular protective activity selected from the group consisting of protecting, maintaining, enhancing or restoring the function or viability of a responsive mammalian cell, tissue or organ.
2. (Withdrawn) The recombinant tissue protective cytokine of claim 1, comprising one or more altered amino acid residue between position 11 to 15 of SEQ ID NO:10 [SEQ ID NO:1], position 44 to 51 of SEQ ID NO 10 [SEQ ID NO:2], position 100-108 of SEQ ID NO [SEQ ID NO:3], or position 146-151 of SEQ ID NO 10 [SEQ ID NO:4].
3. (Withdrawn) The recombinant tissue protective cytokine of claim 1, comprising an altered amino acid residue at one or more of the following positions of SEQ ID NO: 10: 7, 20, 21, 29, 33, 38, 42, 59, 63, 67, 70, 83, 96, 126, 142, 143, 152, 153, 155, 156, or 161.
4. (Withdrawn) The recombinant tissue protective cytokine of claim 1, comprising the amino acid sequence of SEQ ID NO: 10 with one or more of the amino acid residue substitutions of SEQ ID NOs: 15-105 and 119.
5. (Withdrawn) The recombinant tissue protective cytokine of claim 1, comprising the amino acid sequence of SEQ ID NO: 10 with a deletion of amino acid residues 44-49 of SEQ ID NO: 10.

6. (Withdrawn) The recombinant tissue protective cytokine of claim 1, comprising, the amino acid sequence of SEQ ID NO: 10 with at least one of the following amino acid residue substitutions of SEQ ID NOs: 106-118.
7. (Withdrawn) The recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6, further comprising a chemical modification of one or more amino acids.
8. (Withdrawn) The recombinant tissue protective cytokine of claim 7, wherein the chemical modification comprises altering the charge of the recombinant tissue protective cytokine.
9. (Withdrawn) The recombinant tissue protective cytokine of claim 8, wherein a positive or negative charge is chemically added to an amino acid residue where a charged amino acid residue is modified to an uncharged residue.
10. (Withdrawn) The recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6, wherein said cytokine is a human erythropoietin mutein.
11. (Withdrawn) The recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6, wherein said cytokine is a human phenylglyoxal erythropoietin mutein.
12. (Withdrawn) The recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6, wherein the responsive mammalian cell comprises a neuronal, muscle, heart, lung, liver, kidney, small intestine, adrenal cortex, adrenal medulla, capillary, endothelial, testis, ovary, endometrial, or stem cell.
13. (Withdrawn) The recombinant tissue protective cytokine responsive mammalian cell of any one of claims 1, 2, 3, 4, 5, or 6, comprising a photoreceptor, ganglion, bipolar, horizontal, amacrine, Mueller, myocardium, pace maker, sinoatrial node, sinus node, atrioventricular node, bundle of His, hepatocyte, stellate, Kupffer, mesangial, goblet, intestinal gland, enteral endocrine, glomerulosa, fasciculate, reticularis, chromaffin, pericyte,

Leydig, Sertoli, sperm, Graffian follicles, primordial follicles, endometrial stroma, and endometrial cell.

14. (Withdrawn) The recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6, wherein said cytokine is capable of traversing an endothelial cell barrier.

15. (Withdrawn) The recombinant tissue protective cytokine of claim 14, wherein the endothelial cell barrier comprises the blood-brain barrier, the blood-eye barrier, the blood testes barrier, the blood-ovary barrier, and the blood-uterus barrier.

16. (Withdrawn) The recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6, wherein said cytokine is selected from the group consisting of:

- i. a cytokine having a reduced number or no sialic acid moieties;
- ii. a cytokine having a reduced number or no N-linked or O-linked carbohydrates;
- iii. a cytokine having at least a reduced carbohydrate content by virtue of treatment of native cytokine with at least one glycosidase;
- iv. a cytokine having at least one or more oxidized carbohydrates;
- v. a cytokine having at least one or more oxidized carbohydrates and is chemically reduced;
- vi. a cytokine having at least one or more modified arginine residues;
- vii. a cytokine having at least one or more modified lysine residues or a modification of the N-terminal amino group of a cytokine molecule;
- viii. a cytokine having at least a modified tyrosine residue;
- ix. a cytokine having at least a modified aspartic acid or glutamic acid residue;
- x. a cytokine having at a modified tryptophan residue;
- xi. a cytokine having at least one amino acid group removed;
- xii. a cytokine having at least one opening of at least one of the cystine linkages in the cytokine molecule;
- xiii. a truncated cytokine;
- xiv. a cytokine having at least one polyethylene glycol molecule attached;
- xv. a cytokine having at least one fatty acid attached;

- xvi. a cytokine having a non-mammalian glycosylation pattern by virtue of the expression of a recombinant cytokine in non-mammalian cells; and
- xvi. a cytokine having at least one histidine tagged amino acid to facilitate purification.

17. (Withdrawn) The recombinant tissue protective cytokine of claim 16 wherein said cytokine is an asialoerythropoietin.
18. (Withdrawn) The recombinant tissue protective cytokine of claim 17, wherein said asialoerythropoietin is human asialoerythropoietin.
19. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine is hyposialylated or hypersialylated.
20. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 sialic acid moieties.
21. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine comprises more than the fourteen sialic acid moieties present in native erythropoietin.
22. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine is an erythropoietin with no N-linked carbohydrates.
23. (Withdrawn) The recombinant tissue protective cytokine of claim 22, wherein said cytokine is an erythropoietin with no O-linked carbohydrates.
24. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine is treated with at least one glycosidase.
25. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine is periodate-oxidized erythropoietin.

26. (Withdrawn) The recombinant tissue protective cytokine of claim 25, wherein said periodate-oxidized erythropoietin is chemically reduced with sodium cyanoborohydride.
27. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine comprises an R-glyoxal moiety on the one or more arginine residues, wherein R is aryl or alkyl moiety.
28. (Withdrawn) The recombinant tissue protective cytokine of claim 27, wherein said cytokine is phenylglyoxal-erythropoietin.
29. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine is an erythropoietin in which an arginine residue is modified by reaction with a vicinal diketone selected from the group consisting of 2,3-butanedione and cyclohexanedione.
30. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine is an erythropoietin in which an arginine residue is reacted with 3-deoxyglucosone.
31. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine is a molecule having at least one biotinylated lysine or N-terminal amino group.
32. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine is a glucitolyl lysine erythropoietin or fructosyl lysine erythropoietin.
33. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine comprises at least one carbamylated lysine residue.
34. (Withdrawn) The recombinant tissue protective cytokine of claim 33, wherein said carbamylated cytokine is comprised of alpha-N-carbamoylerythropoietin; N-epsilon-carbamoylerythropoietin; alpha-N-carbamoyl, N-epsilon-carbamoylerythropoietin; alpha-N-carbamoylasialoerythropoietin; N-epsilon-carbamoylasialoerythropoietin; alpha-N-carbamoyl, N-epsilon-carbamoylasialoerythropoietin; alpha-N-

carbamoylhyposialoerythropoietin; N-epsilon-carbamoylhyposialoerythropoietin; and alpha-N-carbamoyl, N-epsilon-carbamoylhyposialoerythropoietin.

35. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine comprises at least one acylated lysine residue.

36. (Withdrawn) The recombinant tissue protective cytokine of claim 35, wherein said cytokine comprises at least one acylated lysine residue.

37. (Withdrawn) The recombinant tissue protective cytokine of claim 36, wherein said cytokine comprises at least one acylated lysine residue.

38. (Withdrawn) The recombinant tissue protective cytokine of claim 37, wherein a said acetylated cytokine is comprised of alpha-N-acetylerthropoietin; N-epsilon-acetylerthropoietin; alpha-N-acetyl, N-epsilon-acetylerthropoietin; alpha-N-acetylalaoerythropoietin; N-epsilon-acetylalaoerythropoietin; alpha-N-acetyl, N-epsilon-acetylalaoerythropoietin; alpha-N-acetylhyposialoerythropoietin; N-epsilon-acetylhyposialoerythropoietin; and alpha-N-acetyl, N-epsilon-acetylhyposialoerythropoietin.

39. (Withdrawn) The recombinant tissue protective cytokine of claim 35, wherein a lysine residue of said cytokine is succinylated.

40. (Withdrawn) The recombinant tissue protective cytokine of claim 39, wherein said succinylated cytokine is comprised of alpha-N-succinylerythropoietin; N-epsilon-succinylerythropoietin; alpha-N-succinyl, N-epsilon-succinylerythropoietin; alpha-N-succinylalaoerythropoietin; N-epsilon-succinylalaoerythropoietin; alpha-N-succinyl, N-epsilon-succinylalaoerythropoietin; alpha-N-succinylhyposialoerythropoietin; N-epsilon-succinylhyposialoerythropoietin; and alpha-N-succinyl, N-epsilon-succinylhyposialoerythropoietin.

41. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine comprises at least one lysine residue modified by 2, 4, 6 trinitrobenzenesulfonate sodium or another salt thereof.
42. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine comprises at least one nitrated or iodinated tyrosine residue.
43. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein said cytokine comprises an aspartic acid or glutamic acid residue that is reacted with a carbodiimide followed by reaction with an amine.
44. (Withdrawn) The recombinant tissue protective cytokine of claim 16, wherein a said amine is glycynamide.
45. (Withdrawn) An isolated nucleic acid molecule that comprises a nucleotide sequence which encodes a polypeptide comprising the recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6.
46. (Withdrawn) A vector comprising a nucleic acid molecule of claim 45.
47. (Withdrawn) An expression vector comprising a nucleic acid molecule of claim 45 and at least one regulatory region operably linked to the nucleic acid molecule.
48. (Withdrawn) The vector of claim 46 or 47 that is a pCiNeo vector.
49. (Withdrawn) A genetically-engineered cell which comprises a nucleic acid molecule of claim 45.
50. (Withdrawn) A cell comprising the expression vector of claim 45.
51. (Withdrawn) A pharmaceutical composition comprising a recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6, lacking at least one activity selected from the group consisting of increasing hematocrit, vasoactive action,

hyperactivating platelets, pro-coagulant activities and increasing production of thrombocytes, the cytokine having at least one responsive cellular protective activity selected from the group consisting of protecting, maintaining, enhancing or restoring the function or viability of a responsive mammalian cell, tissue or organ.

52. (Withdrawn) The pharmaceutical composition of claim 51, formulated for oral, intranasal, or parenteral administration.

53. (Withdrawn) The pharmaceutical composition of claim 51, formulated as a perfusate solution.

54. (Currently amended) A method for protecting, maintaining or enhancing the viability of a responsive cell, a tissue comprising a responsive cell, or an organ comprising a responsive cell, wherein said cell, tissue or organ is isolated from a mammalian body, comprising exposing said cell, tissue or organ to an effective amount of a pharmaceutical composition comprising a mutein recombinant tissue protective cytokine, wherein said mutein recombinant tissue protective cytokine

(a) comprises the amino acid sequence of SEQ ID NO:10 with a substitution of an amino acid residue at one of more of the following positions:

(i) 11 to 15 [SEQ ID NO:1];

(ii) 44 to 51 [SEQ ID NO:2];

(iii) 100 to 108 [SEQ ID NO:3]; or

(iv) 146 to 151 [SEQ ID NO:4];

(b) has a reduced level of *in vivo* erythropoietic activity compared to native erythropoietin as determined by the exhypoxic polycythemic mouse bioassay; and

(c) has tissue protective activity *in vivo* as determined by the middle cerebral artery occlusion test or *in vitro* as determined by the P19 assay, wherein the viability of the cell, tissue, or organ is protected, maintained, or enhanced.

55. (Currently amended) The method of claim 54, wherein the mutein recombinant tissue protective cytokine ~~does not affect bone marrow~~ is nonerythropoietic.

56. (Currently amended) A The method of claim 54, wherein the mutein for protecting, maintaining or enhancing the viability of a responsive cell, a tissue comprising a responsive cell, or an organ comprising a responsive cell, isolated from a mammalian body comprising exposing said cell, tissue or organ to a pharmaceutical composition comprising a recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6, that lacks at least one activity selected from the group consisting of increasing hematocrit, vasoactive action, hyperactivating platelets, pro-coagulant activity and increasing production of thrombocytes, wherein the viability of the cell, tissue, or organ is protected, maintained, or enhanced.

57. (Currently amended) A method for ~~the~~ protecting against ~~and or~~ preventing a tissue injury ~~as well as or~~ restoring ~~and or~~ rejuvenating tissue ~~and or~~ tissue function in a mammal, comprising exposing said tissue to a an effective amount of a pharmaceutical composition comprising a mutein recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6, wherein said mutein recombinant tissue protective cytokine

- (a) comprises the amino acid sequence of SEQ ID NO:10 with a substitution of an amino acid residue at one of more of the following positions:
 - (i) 11 to 15 [SEQ ID NO:1];
 - (ii) 44 to 51 [SEQ ID NO:2];
 - (iii) 100 to 108 [SEQ ID NO:3]; or
 - (iv) 146 to 151 [SEQ ID NO:4];
- (b) has a reduced level of *in vivo* erythropoietic activity compared to native erythropoietin as determined by the exhypoxic polycythemic mouse bioassay;
and
- (c) has tissue protective activity *in vivo* as determined by the middle cerebral artery occlusion test or *in vitro* as determined by the P19 assay, that lacks at least one activity selected from the group consisting of increasing hematocrit, vasoactive action, hyperactivating platelets, pro-coagulant activity and increasing production of thrombocytes, wherein the method results in protection against and prevention of a tissue injury as well as restoration and rejuvenation of tissue and tissue function in a mammal.

58. (Currently amended) The method of claim 57, wherein the mammal has or is at risk for cognitive dysfunction, a seizure disorder, chronic seizure disorder, epilepsy, convulsions, nerve root compression, myotonic dystrophy, muscular dystrophy, multiple sclerosis, stroke, hypotension, cardiac arrest, central nervous system injury, neuronal loss, ischemia, subdural hematoma, subarachnoid bleeds, aneurysm, aneurysmal bleeds, myocardial infarction, inflammation, age-related loss of cognitive function, radiation damage, chemotherapy damage, radiotherapy damage, whole brain irradiation damage, cerebral palsy, cerebral supranuclear palsy, progressive supranuclear palsy, neurodegenerative disease, Alzheimer's disease, Parkinson's disease, Huntington's disease, Tourette's syndrome, Leigh disease, Guillain Barre, dementia, AIDS dementia, senile dementia, Lewy body dementia, memory loss, amyotrophic lateral sclerosis, alcoholism, a neuropsychiatric or neuropsychological disorder, mood disorder, anxiety disorder, anxiety, schizophrenia, schizoaffective disorder, obsessive-compulsive disorder, panic disorder, uni-polar affective disorder, depression, major depressive disorder, dysthymic disorder, mania, bi-polar affective disorder, attention deficit disorder, attention deficit hyperactivity disorder, autism, a prion disease, Creutzfeldt-Jakob Creutzfeldt-Jakob disease, Friedrich's ataxia, Wilson's disease, trauma, concussive injury, brain or spinal cord trauma or ischemia, heart-lung bypass, neurological defects from heart-lung bypass, post-operative cognitive dysfunction, embolic injury, hypoxia, mitochondrial dysfunction, abdominal aortic surgery, heart injury, myocardium injury, heart trauma, chronic heart failure, eye tissue damage, macular degeneration, diabetic neuropathy, diabetic retinopathy, glaucoma, retinal ischemia, ~~or~~ retinal trauma, retinitis pigmentosa, optic nerve damage, retinal detachment, arteriosclerotic retinopathy, hypertensive retinopathy, retinal artery blockage, retinal vein blockage, hypotension, a condition associated with hypoglycemia or diabetes, diabetes mellitus, nephrotic symptoms, acute renal failure, or hepatitis.

59. (Withdrawn) A method for facilitating the transcytosis of a molecule across an endothelial cell barrier in a mammal comprising administration to said mammal a composition comprising said molecule in association with a recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6, lacking at least one activity selected from the group consisting of increasing hematocrit, increasing blood pressure, hyperactivating platelets, and increasing production of thrombocytes.

60. (Withdrawn) The method of claim 59, wherein said association is a labile covalent bond, a stable covalent bond, or a non-covalent association with a binding site for said molecule.
61. (Withdrawn) The method of claim 59, wherein said endothelial cell barrier is selected from the group consisting of the blood-brain barrier, the blood-eye barrier, the blood-testis barrier, the blood-ovary barrier, the blood-heart barrier, the blood-kidney barrier, and the blood-placenta barrier.
62. (Withdrawn) The method of claim 59, wherein said molecule is a receptor agonist or antagonist hormone, a neurotrophic factor, an antimicrobial agent, an antiviral agent, a radiopharmaceutical, an antisense oligonucleotide, an antibody, an immunosuppressant, a dye, a marker, or an anti-cancer drug.
63. (Withdrawn) A composition for transporting a molecule via transcytosis across an endothelial cell barrier comprising said molecule in association with a recombinant tissue protective cytokine, of any one of claims 1, 2, 3, 4, 5, or 6, lacking at least one activity selected from the group consisting of increasing hematocrit, vasoactive action, hyperactivating platelets, pro-coagulant activity and increasing production of thrombocytes.
64. (Withdrawn) The composition of claim 63, wherein said association is a labile covalent bond, a stable covalent bond, or a non-covalent association with a binding site for said molecule.
65. (Withdrawn) The composition of claim 63, wherein said molecule is a receptor agonist or antagonist hormone, a neurotrophic factor, an antimicrobial agent, a radiopharmaceutical, an antisense oligonucleotide, an antibody, an immunosuppressant, a dye, a marker, or an anti-cancer drug.
66. (Withdrawn) Use of a recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6, lacking at least one activity selected from the group consisting of increasing

hematocrit, vasoactive action, hyperactivating platelets, pro-coagulant activities and increasing production of thrombocytes.

67. (Withdrawn) The use of claim 66, wherein said association is a labile covalent bond, a stable covalent bond, or a non-covalent association with a binding site for said molecule.

68. (Withdrawn) The use of claim 66, wherein said molecule is a receptor agonist or antagonist hormone, a neurotrophic factor, an antimicrobial agent, a radiopharmaceutical, an antisense oligonucleotide, an antibody, an immunosuppressant, a dye, or a marker, or an anti-cancer drug.

69. (Currently amended) The method of claim 57, wherein the mutein recombinant tissue protective cytokine is administered to the mammal prior to a surgical procedure.

70. (Previously presented) The method of claim 69, wherein the surgical procedure is cardiopulmonary bypass surgery.

71. (New) A method for protecting, maintaining or enhancing the viability of a responsive cell, a tissue comprising a responsive cell, or an organ comprising a responsive cell, wherein said cell, tissue or organ is isolated from a mammalian body, comprising exposing said cell, tissue or organ to an effective amount of a pharmaceutical composition comprising a mutein recombinant tissue protective cytokine, wherein said mutein recombinant tissue protective cytokine

- (a) comprises an amino acid sequence comprising amino acid positions 1 to 10, 16 to 43, 52 to 99, 109 to 145, and 152 to 166 of SEQ ID NO:10;
- (b) has a reduced level of *in vivo* erythropoietic activity compared to native erythropoietin as determined by the exhypoxic polycythemic mouse bioassay; and
- (c) has tissue protective activity *in vivo* as determined by the middle cerebral artery occlusion test or *in vitro* as determined by the P19 assay.

72. (New) A method for protecting against or preventing a tissue injury or restoring or rejuvenating tissue or tissue function in a mammal comprising exposing said tissue to an effective amount of a pharmaceutical composition comprising a mutein recombinant tissue protective cytokine, wherein said mutein recombinant tissue protective cytokine

- (a) comprises an amino acid sequence comprising amino acid positions 1 to 10, 16 to 43, 52 to 99, 109 to 145, and 152 to 166 of SEQ ID NO:10;
- (b) has a reduced level of *in vivo* erythropoietic activity compared to native erythropoietin as determined by the exhypoxic polycythemic mouse bioassay; and
- (c) has tissue protective activity *in vivo* as determined by the middle cerebral artery occlusion test or *in vitro* as determined by the P19 assay.

73. (New) The method of claim 72, wherein the mammal has or is at risk for cognitive dysfunction, a seizure disorder, chronic seizure disorder, epilepsy, convulsions, nerve root compression, myotonic dystrophy, muscular dystrophy, multiple sclerosis, stroke, hypotension, cardiac arrest, central nervous system injury, neuronal loss, ischemia, subdural hematoma, subarachnoid bleeds, aneurysm, aneurysmal bleeds, myocardial infarction, inflammation, age-related loss of cognitive function, radiation damage, chemotherapy damage, radiotherapy damage, whole brain irradiation damage, cerebral palsy, cerebral supranuclear palsy, progressive supranuclear palsy, neurodegenerative disease, Alzheimer's disease, Parkinson's disease, Huntington's disease, Tourette's syndrome, Leigh disease, Guillain Barre, dementia, AIDS dementia, senile dementia, Levy body dementia, memory loss, amyotrophic lateral sclerosis, alcoholism, a neuropsychiatric or neuropsychological disorder, mood disorder, anxiety disorder, anxiety, schizophrenia, schizoaffective disorder, obsessive-compulsive disorder, panic disorder, uni-polar affective disorder, depression, major depressive disorder, dysthymic disorder, mania, bi-polar affective disorder, attention deficit disorder, attention deficit hyperactivity disorder, autism, a prion disease, Creutzfeldt-Jakob disease, Friedrich's ataxia, Wilson's disease, trauma, concussive injury, brain or spinal cord trauma or ischemia, heart-lung bypass, neurological defects from heart-lung bypass, post-operative cognitive dysfunction, embolic injury, hypoxia, mitochondrial dysfunction,

abdominal aortic surgery, heart injury, myocardium injury, heart trauma, chronic heart failure, eye tissue damage, macular degeneration, diabetic neuropathy, diabetic retinopathy, glaucoma, retinal ischemia, retinal trauma, retinitis pigmentosa, optic nerve damage, retinal detachment, arteriosclerotic retinopathy, hypertensive retinopathy, retinal artery blockage, retinal vein blockage, hypotension, a condition associated with hypoglycemia or diabetes, diabetes mellitus, nephrotic symptoms, acute renal failure, or hepatitis.

REMARKS

By this amendment, the specification has been amended to correct minor clerical errors and to incorporate the Substitute Sequence Listing submitted herewith.

In particular, the paragraph at p. 20, ll. 3-17 of the specification has been amended to correct the spelling of the term "Creutzfeldt-Jakob"; the paragraph at p. 47, ll. 11-22 of the specification has been amended for clarity and to correct spelling errors; the paragraph at p. 68, ll. 6-25 of the specification has been amended to correct the spelling of the term "Friedrich's"; and the table at p. 71, l. 13 has been amended to correct the spelling of the term "Guillain Barre."

In addition, the paragraphs at p. 9, l. 27 to p. 10, l. 5 and at p. 42, ll. 1-23 of the specification have been amended to correct typographical errors and to update the reference to the U.S. patent application that was identified as Attorney Docket No. KW00-009C02-US to state that it has been accorded Application Serial No. 10/188,905. A copy of the filing receipt for Application Serial No. 10/188,905 is attached as Exhibit A. As such, no new matter has been added with this amendment. See M.P.E.P. 608.01(p).

The specification at p. 42, ll. 1-23 has also been amended to add the language "in which *in vivo* biological activity was determined by the polycythemic mouse bioassay." This amendment is supported by the application as originally filed at p. 42, ll. 10-11, which incorporates by reference Satake *et al*; 1990, *Biochim. Biophys. Acta* 1038:125-9 in its entirety for its description of molecular biological techniques for generating erythropoietin derivatives and testing them for *in vivo* biological activity of erythrocytes (*i.e.*, erythropoietic activity) using the polycythemic mouse bioassay. See Satake, page 126, col. 2: "in *in vivo* biological activity was determined by the exhypoxic polycythemic mouse bioassay," citing Cotes and Bangham, 1961, *Nature* 191:1065-1067 (reference C102 of record in the subject application). As such, no new matter has been added with this amendment. See M.P.E.P. 608.01(p).

Lastly, sequence identifiers were inserted into the paragraphs at p. 39, l. 8 to p. 40, l. 14 and p. 89, ll. 14-20. In the paragraphs at p. 90, ll. 4-10, the sequence identifier for full-length erythropoietin (EPO) has been amended from SEQ ID NO:10 to SEQ ID NO:213.

Claims 1-70 were pending in the instant application. Claims 1-53, 59-68, and 69-70 are withdrawn from consideration. By this amendment, claims 54-58 are amended and new claims 71-73 are added, for the reasons discussed below.

Claims 54 and 57 have been amended to specify that the mutein recombinant tissue protective cytokine (alternatively referred to herein as "EPO mutein") comprises the amino acid sequence of SEQ ID NO:10 with a substitution of an amino acid residue at one or more of the following positions: (i) 11 to 15 [SEQ ID NO:1]; (ii) 44 to 51 [SEQ ID NO:2]; (iii) 100 to 108 [SEQ ID NO:3]; or (iv) 146 to 151 [SEQ ID NO:4]. Support for this amendment may be found in the claims and specification as filed, *inter alia*, at p. 4, ll. 20 to p. 5, l. 4; p. 6, ll. 15-16; p. 6, l. 22 to p. 9, l. 20; p. 40, ll. 3-7; Example 3 starting at p. 89, particularly p. 90, ll. 4-10 and p. 101, l. 19 to p. 102, l. 24; Example 15 at pp. 124-125; Example 16 at pp. 125-126; and Example 18 at pp. 128-129; the Substitute Sequence Listing for the corresponding sequences referred to in the aforementioned pages; and claim 2 as originally filed. Claims 54 and 57 have also been amended to recite specific tests for determining erythropoietic and tissue protective activity. In particular, the recited muteins have: (i) a reduced level of *in vivo* erythropoietic activity compared to native erythropoietin as determined by the exhypoxic polycythemic mouse bioassay, and (ii) tissue protective activity *in vivo* as determined by the middle cerebral artery occlusion test or *in vitro* as determined by the P19 assay. Support for this amendment may be found in the specification *inter alia* at p. 42, paragraph beginning at l. 1 as amended herein; Example 3 at p. 103, l. 18 to p. 104, l. 3; and Example 15 at pp. 124-125. In addition, claims 54 and 57 have been amended to recite that "an effective amount" of a pharmaceutical composition is administered. Support for this amendment may be found in the specification, *e.g.*, at p. 19, ll. 1-19.

Claim 58 has been amended to add the term "is at risk for." Support for this amendment may be found in the specification at, *inter alia*, p. 20, ll. 3-8; p. 24, ll. 16-19; p. 31, ll. 26-30; p. 64, ll. 13-15 and ll. 29-32; p. 79, ll. 19-25; and p. 80, ll. 6-15. The listing of indications in claim 58 has been amended to more distinctly point out the invention. Support for this amendment may be found in the specification, *e.g.*, at p. 20, ll. 3-17; p. 31, ll. 21-30; p. 66, l. 25 to p. 68, l. 32; the Table at pp. 69-71; and p. 79, ll. 14-25. Claim 58 has also been amended to correct the spelling of Creutzfeldt-Jakob disease.

Claims 54 and 57 have also been amended to correct minor typographical and/or grammatical errors and to clarify the invention. Claim 56 has been amended to depend from claim 54. Claim 69 has been amended so that it is consistent with antecedent claim 57 as amended.

Support for new claims 71-73 may be found in the specification and claims as filed, *inter alia*, at p. 4, ll. 20 to p. 5, l. 4; p. 6, ll. 15-16; p. 6, l. 22 to p. 9, l. 20; p. 19, ll. 1-19; p. 20, ll. 3-17; p. 24, ll. 16-19; p. 31, ll. 21-30; p. 40, ll. 3-7; p. 42, paragraph beginning at l. 1 as amended herein; p. 64, ll. 13-15 and ll. 29-32; p. 66, l. 25 to p. 68, l. 32; the Table at pp. 69-71; p. 79, ll. 14-25; p. 80, ll. 6-15; Example 3 starting at p. 89, particularly p. 90, ll. 4-10 and p. 101, l. 19 to p. 102, l. 24; Example 15 at pp. 124-125; Example 16 at pp. 125-126; and Example 18 at pp. 128-129; the Substitute Sequence Listing for the corresponding sequences referred to in the aforementioned pages; and claim 2 as originally filed.

No new matter has been added by this amendment. Thus, claims 1-73 will be pending upon entry of the present amendment, and claims 54-58 and 69-73 will be under examination.

I. STATEMENT OF THE SUBSTANCE OF THE INTERVIEW

Applicants thank Primary Examiner Aditi Dutt and Supervisory Patent Examiner Jeffrey Stucker for the courtesies extended during the interview of February 6, 2008 at the United States Patent and Trademark Office ("the Interview"). Also present at the Interview were Drs. Anthony Cerami and Michael Brines, two of the inventors of the instant application, Frederick J. Hamble, Esq., of Warren Pharmaceuticals, Inc., Mary Catherine DiNunzio, Esq., of H. Lundbeck A/S, and Applicants' representatives Laura A. Coruzzi, Esq., Eileen E. Falvey, Esq., and Tracy J. LaGrassa, Ph.D., of Jones Day.

During the Interview an overview of the invention was presented. In summary, the inventors described their discovery that EPO provides tissue protective activity via a pathway distinct from the pathway it uses for erythropoiesis, *i.e.*, red blood cell production. EPO exerts its erythropoietic effect via an EpoR homodimer (the "Classical EPO Receptor"), whereas EPO's tissue protective activity is mediated through its interaction with a different receptor, referred to herein as the "Tissue Protective Receptor Complex," which is a

heteromer of EpoR and the beta common receptor (β cR). EPO signaling through the Classical EPO Receptor (present predominantly on blood forming cells) elicits an increase in erythrocytes, platelets, and blood pressure. In contrast, activation by EPO of the Tissue Protective Receptor Complex (present on many tissues) leads to a wider range of tissue protective effects. Based on the inventors' discovery that EPO can cross tight endothelial cell barriers, they found that exogenously administered EPO is capable of conferring tissue protection on any tissue that expresses the Tissue Protective Receptor Complex. Therefore, as a tissue protective cytokine, EPO counteracts the tissue damage at the root of a wide variety of diseases and disorders. For example, the inventors found that EPO protects against tissue damage caused by the pro-inflammatory cytokine TNF α , reduces the extent of TNF α -induced damage, and promotes healing and regeneration of affected tissues. Thus, the ability of EPO to prevent cell death and tissue damage that is common to many disease conditions demonstrates the widespread therapeutic implications of EPO and EPO muteins.

The inventors found that modified EPOs that cannot bind to the Classical EPO Receptor and stimulate red blood cell production were still effective in their tissue protective functions. Such modified EPOs have the benefit of providing tissue protection without the effect of increasing erythropoiesis, and therefore are suitable for high dose administration or chronic administration often required for tissue protection – for example, for administration to stroke victims – while avoiding the risk of thrombosis associated with administration of wild-type EPO. This concept was demonstrated with *in vitro* and *in vivo* experiments that showed that EPO muteins, particularly those with amino acid substitutions in the domains needed for binding to the Classical EPO Receptor, and EPOs in which the amino acid side chains have been masked by chemical modifications, are both nonerythropoietic and tissue protective in various animal disease models.

The outstanding rejections under 35 U.S.C. § 112, first paragraph, for scope of enablement, made in the Office Action dated December 31, 2007 (the "Office Action") were discussed. Because the Tissue Protective Receptor Complex is present on a large variety of tissues, and EPO muteins with reduced erythropoietic function are tissue protective in a broad range of diseases and injuries, EPO muteins are able to confer tissue protection in a broad range of tissues and for a broad range of diseases and injuries. The possibility of amending

the claims to recite EPOs with mutations in specific regions that affect binding to the classical EPO-R homodimers was discussed.

In view of this discussion, the Examiners indicated that claims reciting EPO muteins having the structural characteristics of having mutations in the regions needed for receptor homodimer binding but not in the remainder of the molecule and the functional characteristics of exhibiting tissue protective activity would be considered if excessive searches were not required.

The Examiners also indicated that they might reconsider the species election set forth in the restriction requirement, mailed June 20, 2006. Specifically, the Examiners indicated that they would consider examining the claims with respect to a greater number of EPO muteins, each mutein capable of binding the Tissue Protective Receptor Complex but not the Classical EPO Receptor, as long as excessive further searches of EPO sequences would not be required.

Applicants also presented results of experiments to support enablement commensurate with the full breadth of the limitation "protecting against and preventing a tissue injury" as well as "restoring and rejuvenating tissue and tissue function in a mammal." The Examiners indicated that the evidence and arguments would be considered if presented.

This Amendment, the evidence presented in the Declaration of Dr. Michael L. Brines, M.D., Ph.D. (the "Brines Declaration") submitted herewith, and the remarks herein reflect the discussion during the Interview.

II. SUBSTITUTE SEQUENCE LISTING

A Substitute Sequence Listing is submitted herewith to correct the Sequence Listing, which lists sequences of the full-length EPO precursor, which includes a leader sequence, whereas the specification discloses amino acid positions of the mature EPO protein, which lacks the leader sequence.

The Substitute Sequence Listing is amended so that SEQ ID NOs:4, 10, and 15-119 represents mature EPO sequences rather than full-length EPO sequences. SEQ ID NO:6 in the Substitute Sequence Listing now represents the full-length EPO precursor.

No new matter is added by this Substitute Sequence Listing, which is supported by the specification as filed. The specification as filed discloses that the first 27 amino acid residues of full-length EPO is a leader sequence (see p. 90, ll. 4-10) and it was well known in the art that mature EPO is produced by the removal of this 27-amino acid leader sequence. See, e.g., Jacobs K *et al.* 1985. "Isolation and characterization of genomic and cDNA clones of human erythropoietin," *Nature* 313(6005):806-810; reference C173 in the Supplemental Information Disclosure Statement submitted concurrently herewith. The specification, however, refers to amino acid positions in mature EPO, but misidentifies them with sequence identifiers that represent full-length EPO (see, e.g., p. 4, ll. 24-25; p. 90, ll. 4-10; and SEQ ID NO:10 of the specification as filed, which states that the sequence TKVNFYAW is at amino acid positions 44-51, but shows that this sequence is located in full-length EPO at positions 71-78). Thus, one of skill in the art reading the specification would understand that the EPO amino acid positions in the specification refer to mature EPO. Therefore, no new matter has been added with the Substitute Sequence Listing.

The Substitute Sequence Listing also contains a correction to the listing of the sequences in response to the Examiner's objection that SEQ ID NO:62 and SEQ ID NO:5 are the same. The sequence identified by SEQ ID NO:5 has been replaced with the sequence formerly identified as SEQ ID NO:212. Applicants note that SEQ ID NO:6 and SEQ ID NO:44 are also identical in the Sequence Listing as filed. In order to correct this duplication, the sequence identified by SEQ ID NO:6 has been replaced with the sequence formerly identified as SEQ ID NO:10 (full-length EPO) in the Substitute Sequence Listing.

III. REQUEST FOR RECONSIDERATION OF RESTRICTION REQUIREMENT AND SPECIES ELECTION

As currently amended, claims 54 and 57 and their dependent claims thereon are limited with respect to the number of mutein recombinant tissue protective cytokines (hereinafter referred to as "EPO muteins") that fall within their scope. In view of this amendment, Applicants respectfully request that the Examiner reconsider the finality of the restriction requirement and the species election of a single EPO point mutation. In particular, Applicants request consideration of the full scope of claims 54-58 as amended, and request

that the Examiner withdraw the objection to claims 56-58 as allegedly reciting non-elected subject matter.

Applicants also traverse the Examiner's withdrawal of claims 69 and 70, and request reconsideration of their withdrawal. Applicants submit that these claims are within the scope of their antecedent claim 57. Specifically, claim 57 as currently amended recites a method for "protecting against or preventing a tissue injury" using an EPO mutein. Claims 69 and 70 recite that the EPO mutein is administered before a surgical procedure, which is a specific embodiment of the method of claim 57. See specification *inter alia* at p. 31, ll. 21-30 and p. 64, ll. 17-32. Accordingly, the subject matter of claims 69 and 70 is within the scope of claim 57 and therefore within the scope of the elected subject matter. As such, it is believed that claims 69 and 70 would not require additional searches. Applicants therefore respectfully request that the Examiner consider claims 69 and 70.

IV. THE REJECTIONS UNDER 35 U.S.C. § 112, FIRST PARAGRAPH, FOR LACK OF ENABLEMENT, SHOULD BE WITHDRAWN

Claims 54-58 are rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement. In particular, the Examiner contends that the specification does not reasonably provide enablement across the full scope of the claims. The Examiner acknowledges that the art recognizes protective effects of four different EPO muteins in ischemic injury models and certain inflammatory conditions, but contends that the art and the specification fail to provide guidance or sufficient scientific basis to use any tissue protective cytokine for any tissue injury (Office Action, p. 5). The Examiner concludes that undue experimentation would be required to practice the claimed invention based on the application of the *Wands* factors: "breadth of the claims encompassing the use of molecules with no precise structural requirements, the lack of adequate guidance . . . supporting a therapeutic effect of EPO molecules on the broadly claimed chronic and/or degenerative diseases or disorders, or guidance on their use, the unpredictability in the art . . . and the complex nature of the invention" (Office Action, ¶ bridging pp. 8-9).

Applicants believe that amended claims 54-58 and new claims 71-73 are enabled over their entire scope. Each of these claims recites a method comprising exposing cells, tissue, or organs to a particular EPO mutein. One of skill in the art can readily practice the claimed

invention over its entire scope without undue experimentation. The claims recite a defined class of EPO muteins with amino acid substitutions at a limited number of amino acid residues. The claims further provide a clear-cut test that can be used by the skilled artisan to determine whether any particular EPO mutein within this defined class has the required activity, *i.e.*, tissue protection but reduced erythropoietin activity. Thus, a skilled person can readily determined the limited number of EPO muteins encompassed by the claims, and use them to practice the invention without undue experimentation.

1. **EPO muteins**

Claims 54-58 have been amended to recite methods using specific EPO muteins with amino acid substitutions in four regions that affect binding to the Classical EPO Receptor, *i.e.*, amino acids at positions 11 to 15 of SEQ ID NO:10 [SEQ ID NO:1]; 44 to 51 of SEQ ID NO:10 [SEQ ID NO:2]; 100 to 108 of SEQ ID NO:10 [SEQ ID NO:3]; and 146 to 151 of SEQ ID NO:10 [SEQ ID NO:4] (see specification at, *e.g.*, p. 40, II. 2-7). Because these EPO muteins have amino acid substitutions in regions that affect binding to the Classical EPO Receptor, they have reduced erythropoietic activity. However, since the amino acids in these regions do not affect binding to the Tissue Protective Receptor Complex, these EPO muteins maintain their tissue protective function. See Brines Declaration, ¶¶ 23-24.

The claims have also been amended to recite specific, art-recognized tests that require that the EPO muteins have reduced erythropoietic activity yet maintain tissue protective activity. In particular, the claims require that the EPO muteins have (i) a reduced level of *in vivo* erythropoietic activity compared to native erythropoietin as determined by the exhypoxic polycythemic mouse bioassay and (ii) tissue protective activity *in vivo* as determined by the middle cerebral artery occlusion test or *in vitro* as determined by the P19 assay.

As such, the claims as amended recite a limited class of EPO muteins having structural and functional features necessary to provide tissue protection while having a reduced ability to stimulate erythropoiesis and are not directed to any tissue protective cytokine.

The amended and new claims are enabled by the specification's extensive teaching of methods to make the claimed EPO muteins and test them for erythropoietic and tissue protective activity. See Brines Declaration, ¶¶ 5 and 18-22. Additional such assays were well-known in the art as of the filing date of the instant application. See Brines Declaration, *id.* Indeed, several such muteins have been made and tested for erythropoietic activity and tissue protective activity in accordance with these teachings, as demonstrated by the working examples of the specification and experiments conducted after the filing date of the instant application (see explained in the Brines Declaration, ¶¶ 5-12), as described below.

Amino acids 100-108. As examples of EPO muteins with amino acid substitutions at positions 100 to 108 of SEQ ID NO:10 [SEQ ID NO:3], the specification provides working examples that show that EPO muteins with substitutions in this region are tissue protective and have reduced erythropoietic activity compared to wild-type recombinant EPO. For example, the EPO muteins S100E and R103E have reduced erythropoietic activity (see specification, Example 17 at p. 126, l. 12 to p. 128, l. 13) but are tissue protective. In particular, the specification teaches that S100E maintains the viability of neuroblastoma cells against rotenone in an *in vitro* assay (Example 3 at page 102, ll. 5-23). The specification further teaches that S100E is protective in well-established assays for apoptosis, for example, protection of P19 cells from apoptosis following serum withdrawal (Example 15 at p. 124, l. 1 to p. 125, l. 2), protection of primary hippocampal neuron cell cultures from NMDA-induced cell death (Example 14 at p. 122, ll. 30 to p. 123, l. 30), and protection of neuronal-like cells from cell death upon withdrawal of nerve growth factor (Example 16 at p. 125, l. 5 to p. 126, l. 6). See also Brines Declaration, ¶¶ 8-9. In addition, the mutein R103E has been shown to reduce NMDA-induced apoptosis of rat hippocampal neurons *in vitro*. See Brines Declaration, ¶ 9. Thus, in well-established *in vitro* assays for apoptosis using different cell types, muteins substituted in amino acid positions 100-108 were shown to be cell-protective.

These *in vitro* results have been corroborated by *in vivo* studies. Example 12 of the specification shows that the S100E mutein reduces functional deficits in motor neurological function in an animal model of traumatic spinal cord injury (specification at p. 115, ll. 21-30 and p. 116, l. 20 to p. 117, l. 5). The S100E mutein and the R103E mutein are tissue protective in retina, shown using an animal model of glaucoma. See Example 18 of the

specification at p. 128, l. 17 to p. 129, l. 8. See also Brines Declaration, ¶ 10. In a more recent study, the mutein S100E was demonstrated to improve neurological function after stroke. In another experiment, S100E and R103E were found to prevent injury that was caused by sciatic nerve compression in rats. See Brines Declaration, ¶¶ 11-12.

The specification also teaches that the double substitution EPO mutein K45D/S100E is tissue-protective in an *in vivo* animal model of glaucoma. See Example 18 of the specification at p. 128, l. 17 to p. 129, l. 8. Moreover, the double substitution mutein K45D/S100E has been demonstrated to prevent injury due to sciatic nerve compression in rats. See Brines Declaration, ¶ 12.

Amino acids 146-151. As an example of an EPO mutein with amino acid substitutions at positions 146 to 151 of SEQ ID NO:10 [SEQ ID NO:4], the specification teaches that the EPO mutein R150E prevents NMDA-induced cell death in primary hippocampal neuron cell cultures. See specification, Example 14 at p. 122, ll. 30 to p. 123, l. 30. Moreover, in a subsequent study, R150E was found to prevent P19 cell apoptosis. See Brines Declaration, ¶ 9. Again, these *in vitro* results were confirmed *in vivo*. For instance, the specification provides a working example to demonstrate that R150E is tissue protective in retina in an *in vivo* animal model of glaucoma. See specification, Example 18 at p. 128, l. 17 to p. 129, l. 8.

Chemically-modified amino acids. EPO muteins with amino acid substitutions in four regions that affect binding to the Classical EPO Receptor have reduced erythropoietic activity but maintain their tissue protective function. A similar effect is achieved when the charge of amino acids in these four regions is altered by chemical modification. See Brines Declaration, ¶ 13. Indeed, the specification teaches several chemical modifications of the EPO molecule that result in EPOs with tissue protective activity but reduced erythropoietic activity. Such modifications include modification of arginine residues with a vicinal diketone (e.g., cyclohexanedione) or R-glyoxal (e.g., phenylglyoxal), modification of tyrosine residues by nitration (using, e.g., a trinitrophenyl compound) or iodination, modification of lysine residues by, e.g., carbamylation, guanidination, or carboxymethylation, and oxidation of tryptophan residues by succinylation. See specification at p. 52, l. 1 to p. 53, l. 25; p. 54, ll. 8-10; p. 55, ll. 13-18; p. 109, ll. 23-28; Example 4 at p. 104, ll. 11-22 and p. 105, l. 23 to p.

107, l. 10; Example 5 at p. 108, l. 19 to p. 109, l. 16; Example 6 starting at p. 111; Example 12 at p. 117, l. 6 to p. 118, l. 15.

EPOs with chemically-modified lysines (EPOs that have been carbamylated, carboxymethylated, PEGylated, carbamylated and PEGylated, or succinylated), arginines (by cyclohexanedionation and phenylglyoxalation), glutamate/aspartate (EDC-ethanolamination), and tyrosines (trinitrophenylation), and EPOs in which the disulfides are reduced by iodoacetamidation, have been shown to be nonerythropoietic yet tissue protective in various tissues and disease or injury conditions. See Brines Declaration, ¶¶ 13-17.

Accordingly, if an EPO with a chemically-modified arginine, for example, in a domain that affects Classical EPO Receptor binding (*i.e.*, a chemical modification of the arginine at position 14, 103 or 150) retains its tissue protective function, it would follow that an EPO having a single substitution of an arginine at that same position would also be tissue protective. This is indeed the case, as described *supra* for the R103E and R150E muteins. Therefore, the experiments showing a tissue protective function for EPOs with chemically-modified amino acids at particular positions indicate that EPOs with mutations in amino acids at those positions – in particular, amino acids 11-15 of SEQ ID NO:10 (VLQRY), amino acids 44-51 (TKVNFYAW), 100-108 (SGLRSLTTL), and 146-151 (SNFLRG) (residues that have been modified by chemical modification are shown in bold/underline) – are also tissue protective.

2. Protection or Rejuvenation of Tissues

The Examiner acknowledges that routine technology and bioassays can be used to make the claimed EPO muteins, but contends that the testing of numerous mutants for tissue protection would require undue experimentation, because tissue responsiveness will vary depending on the mutein, different mutein doses must be tested, and *in vitro* testing does not parallel the *in vivo* protection, wherein the injury can arise due to various causes (Office Action, p. 8). Applicants respectfully submit that practicing the claimed invention does not require undue experimentation.

The methods of the present claims are used to prevent and treat conditions that result from cell death and subsequent tissue damage induced by inflammation and oxidative

damage. Because the Tissue Protective Receptor Complex is present on a large variety of tissues, and EPO muteins with reduced erythropoietic function are tissue protective in a broad range of diseases and injuries, EPO muteins are able to confer tissue protection in a broad range of tissues and for a broad range of diseases and injuries. Thus, an EPO mutein found to have tissue protective activity in the *in vitro* or *in vivo* assays recited in the claims can be used in the claimed methods regardless of the source of the injury, other symptoms of the disease or condition, and the cell type affected. See Brines Declaration, ¶¶ 23-28.

Therefore, the skilled artisan would be able to practice the claimed invention with a limited amount of experimentation using routine, art-accepted methods. This amount of experimentation is well within the legal standard, which does not preclude a certain amount of experimentation and unpredictability of the results. *In re Wands*, 8 U.S.P.Q.2d 1400, 1404 (Fed. Cir. 1988); *In re Angstadt*, 190 U.S.P.Q. 214, 219 (C.C.P.A. 1976); *In re Neuberger & Rabbitts*, 2002 WL 33952578 (B.P.A.I. 2002)(Board reversed Examiner's non-enablement rejection of claims to a biotechnological invention because "a requirement for certainty would be incompatible with any amount of experimentation and therefore incompatible with the standard of enablement." *Id.* at 3). All that is required is a reasonable amount of guidance with respect to the direction of the experimentation; reasonable certainty with regard to the *outcome* of the experimentation is *not* required.

Moreover, the skilled artisan would understand that a certain amount of experimentation and optimization is required to determine the efficacy (whether using a cell line or experimental animal or in a patient) of a particular EPO mutein. Indeed, the optimal dosage for any pharmaceutical composition must be decided according to the judgment of the practitioner and each patient's circumstances, and the dose is determined based not only on the particular EPO mutein but also on the condition or disease to be treated, the body mass of the patient, the route of administration, *etc.* See specification at p. 77, ll. 8-20. As stated in *In re Brana*, 5 F.3d 1557, 34 U.S.P.Q.2d 1437, 1442 (Fed. Cir. 1995), the testing for the full safety and *effectiveness* of a product is more properly left to the Food and Drug Administration and the requirements under the law for obtaining a patent should not be confused with the requirements for obtaining government approval to market a particular drug or therapeutic method for public use.

Thus, based on the applicable case law and the teachings of the specification, Applicants do not believe that the need to determine the optimal dosage of a mutetin is a proper basis for an allegation of undue experimentation.

Furthermore, Applicants also disagree with the Examiner's contention that *in vitro* testing does not parallel the *in vivo* protection. Indeed, the tissue protection obtained in *in vitro* and *in vivo* assays with specific EPO mutetins described *supra* provides ample evidence to the contrary. Tissue protection *in vitro* is predictive of tissue protection *in vivo*, as demonstrated by the working examples of the instant specification and explained in the Brines Declaration. See Brines Declaration, ¶¶ 18-20.

Finally, the Examiner acknowledges that EPO mutetins spare neuronal tissue loss due to injury and reduce cell death, but states that it would be necessary to "implicate EPO as being able to induce *new cellular growth*, which would be necessary to restore or rejuvenate tissue" (Office Action, ¶ bridging pp. 5-6; emphasis added). Applicants respectfully disagree that a demonstration that EPO can induce new cellular growth is required to "restore" or "rejuvenate" tissue.

The term "restore" in the claims is understood to mean (1): give back, return; and (2): to put or bring back into existence or use (definitions (1) and (2) from Webster's Ninth New Collegiate Dictionary, Springfield, MA: Merriam-Webster, Inc., 1986, p. 1005, entry for "restore" (Exhibit B)). In the context of restoring function, meanings (1) and (2) are appropriate where a mutetin may be used to "give back" function to a cell, tissue, or organ that had lost function, *e.g.*, as a result of injury or trauma, or where a mutetin may be used to put a cell, tissue, or organ "back into use." By analogy, a work of art or a house may be "restored," but it is never fully returned to its original state or condition. Likewise, Applicants submit that the ordinary meaning of the term "restore," as it is used in the instant claims, does not require new cell growth or even that the claimed cells, tissues, and organs are fully returned to their original state or regain full function.

Likewise, "rejuvenate" means: (a) to make young or youthful again: reinvigorate; (b): to restore to an original or new state (definition (1) from Webster's Ninth New Collegiate Dictionary, Springfield, MA: Merriam-Webster, Inc., 1986, p. 994, entry for "rejuvenate")

(Exhibit B)). In the context of rejuvenating tissue or tissue function, this definition is appropriate where a mutein may be used to "reinvigorate" a tissue that had lost function, for example, as a result of injury or trauma, or where a mutein may be used to "restore" (as defined in the preceding paragraph) a tissue – not necessarily to its original state, but to a *new, functional state*. As such, Applicants submit that the ordinary meaning of the term "rejuvenate," as it is used in the instant claims, does not require new cell growth but only that the function of the tissue or organ is restored.

These definitions of restore and rejuvenate are consistent with the teachings of the specification. For example, the specification provides an experiment in which mice were subjected to a brain trauma that impairs cognitive function and then treated with EPO. The mice that received EPO demonstrated an improvement of cognitive function – thus, the treated mice had restoration of brain function. See Example 10 of specification starting at p. 114. Therefore, the meaning of the terms "restore" and "rejuvenate" as used in the specification do not require new cell growth.

Based on the above, claims 54-58 satisfy the enablement requirement. Applicants therefore respectfully request the withdrawal of the rejection of claims 54-58 under 35 U.S.C. § 112, first paragraph, for lack of enablement. Applicants further submit that, for the reasons set forth above, new claims 69-73 are also enabled.

V. THE REJECTIONS UNDER 35 U.S.C. § 112, FIRST PARAGRAPH, WRITTEN DESCRIPTION, SHOULD BE WITHDRAWN

Claims 54-58 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement. Applicants believe that the amendment of claims 54, 56 and 57 obviates this rejection for the reasons set forth below.

Specific mutein recombinant tissue protective cytokines that may be used in the claimed invention are described throughout the specification. For example, particular muteins within the scope of the claims as amended are provided at, e.g., pp. 32-35 and 47-49 of the specification. Examples of specific EPO muteins that are reduced for erythropoietic activity compared to EPO are provided in the working examples of the specification (see Example 17). The tissue protective function of EPO muteins is also demonstrated by

working examples. See specification, Example 3 (S100E), Example 11 (S100E), Example 14 (R130E and R150E), Example 15 (S100E), Example 16 (S100E), and Example 18 (R103E, R150E, S100E).

Moreover, the instant specification discloses a correlation between the structure of the claimed muteins and their function. Specifically, the regions now defined in the claims affect erythropoietic activity of EPO. Mutations in one or more amino acids in this area yields a mutein having reduced erythropoietic activity compared to native recombinant human EPO. See specification at p. 4, l. 20 to p. 5, l. 2.

Therefore, the specification provides extensive description of the EPO muteins recited in the claims, methods for their use, and numerous working examples that demonstrate the correlation between the structure of the EPO muteins and their function, such that a skilled artisan would recognize that Applicants were in possession of the claimed invention at the time of filing. Thus, the rejection under 35 U.S.C. § 112, first paragraph, for lack of written description should be withdrawn.

VI. THE REJECTIONS UNDER 35 U.S.C. § 102 SHOULD BE WITHDRAWN

Claims 54-58 are rejected under 35 U.S.C. § 102(e), as allegedly being anticipated by International Application Publication No. WO 02/053580 to Brines *et al.*, filed on December 28, 2001. Applicants submit that International Application Publication No. WO 02/053580 does not anticipate the claims as amended because this reference generically discloses EPO muteins, but does not disclose the specific EPO muteins recited in the instant claims. This reference does not list the specific muteins recited in the instant claims, *i.e.*, EPO muteins with the particular structural and functional features required by the claims, such as mutations in 28 amino acids of the 165 amino acids of the mature protein. See specification at p. 39, ll. 9-12. Therefore, the broad genus of "mutein" disclosed in these references does not anticipate the specific, defined mutein species of the claims as amended. *Cf. Impax Laboratories, Inc. v. Aventis Pharmaceuticals Inc.*, 468 F.3d 1366 (Fed. Cir. 2006) (holding that a prior patent containing a formula including hundreds of compounds does not anticipate the specific compound of the claims, and the specific compound was not defined in the prior patent).

Accordingly, amended claims 54-58 are not anticipated by WO 02/053580 and Applicants respectfully request the withdrawal of this rejection.

Claims 54-58 are rejected under 35 U.S.C. § 102(b), as allegedly being anticipated by Campana *et al.*, 1998, *Int J Mol Med* 1(1):235041; abstract ("Campana"). Applicants believe that this rejection is in error. The EPO muteins of amended claims 54-58 require a substitution of an amino acid residue at one or more specific amino acid positions of the EPO protein. In contrast, Campana discloses a 17-mer EPO peptide, which could be generated by deletion of amino acids from the EPO sequence. Since the claims recite EPO proteins with alterations at specific amino acid positions, and the Campana peptide does not have any substituted amino acids, Campana does not anticipate the claims. Moreover, the claimed methods for protecting, maintaining or enhancing the viability of cells, tissues, or organs, treating or preventing diseases, and restoring or rejuvenating tissues or tissue function are not taught by Campana.

Therefore, Applicants respectfully request the withdrawal of the rejection of claims 54-68 for anticipation by Campana under 35 U.S.C. § 102(b).

**VII. THE CLAIM REJECTIONS FOR DOUBLE PATENTING
SHOULD BE CONTINUED TO BE HELD IN ABEYANCE**

Claim 57 is rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-10 of U.S. Patent No. 6,531,121 B2.

Claims 54-58 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 35 and 37-38 of copending Application No. 10/188,905.

Claims 57-58 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1 and 15 of copending Application No. 09/716,960.

Claims 54-56 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 2 and 5 of copending Application No. 10/351,640.

Claim 57 is provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claim 1 of copending Application No. 10/185,841.

Claim 57 is provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1 and 4 of copending Application No. 10/573,905.

In response Applicants request that the double patenting rejections continue to be held in abeyance until indication of allowable subject matter in the present application.

CONCLUSION

Entry of the foregoing amendments and remarks into the record of the above-identified application is respectfully requested. Applicants estimate that the remarks made herein place the pending claims in condition for allowance.

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Enclosures

Appendix A: Replacement table for pages 69-71 of the specification

<i>Cell, tissue or organ</i>	<i>Dysfunction or pathology</i>	<i>Condition or disease</i>	<i>Type</i>
Heart	Ischemia	Coronary artery disease	Acute, chronic
		Myocardial infarction	Stable, unstable
		Angina	Dressler's syndrome
		Congenital heart disease	Valvular
		Prinzmetal angina	Cardiomyopathy
		Cardiac rupture	Aneurysmatic
		Angitis	Septal perforation
	Arrhythmia	Tachy-, bradyarrhythmia	Stable, unstable
	Congestive heart failure	Supraventricular, ventricular	Hypersensitive carotid sinus node
		Conduction abnormalities	
		Left, right, bi-ventricular, systolic, diastolic	Cardiomyopathies, such as idiopathic familial, infective, metabolic, storage disease, deficiencies, connective tissue disorder, infiltration and granulomas, neurovascular
		Myocarditis	Autoimmune, infective, idiopathic
	Blunt and penetrating trauma	Cor pulmonale	
Vascular	Toxins	Cocaine toxicity	
	Hypertension	Primary, secondary	
	Decompression sickness		
	Fibromuscular hyperplasia		
	Aneurysm	Dissecting, ruptured, enlarging	
Lungs	Obstructive	Asthma	
		Chronic bronchitis, Emphysema and airway obstruction	
	Ischemic lung disease	Pulmonary embolism, Pulmonary thrombosis, Fat embolism	
	Environmental lung diseases		
	Ischemic lung disease	Pulmonary embolism	
	Interstitial lung disease	Pulmonary thrombosis	
		Idiopathic pulmonary fibrosis	
	Congenital	Cystic fibrosis	
	Cor pulmonale		
	Trauma		

<i>Cell, tissue or organ</i>	<i>Dysfunction or pathology</i>	<i>Condition or disease</i>	<i>Type</i>
	Pneumonia and pneumonitides	Infectious, parasitic, toxic, traumatic, burn, aspiration	
	Sarcoidosis		
Pancreas	Endocrine	Diabetes mellitus, type I and II	Beta cell failure, dysfunction Diabetic neuropathy
		Other endocrine cell failure of the pancreas	
	Exocrine	Exocrine pancreas failure	pancreatitis
Bone	Osteopenia	Primary	Hypogonadism immobilisation Postmenopausal Age-related Hyperparathyroidism Hyperthyroidism Calcium, magnesium, phosphorus and/or vitamin D deficiency
		secondary	
	Osteomyelitis		
	Avascular necrosis		
	Trauma		
	Paget's disease		
Skin	Alopecia	Areata	Primary
		Totalis	Secondary Male pattern baldness
	Vitiligo	Localized generalized	Primary secondary
	Diabetic ulceration		
	Peripheral vascular disease		
	Burn injuries		
Autoimmune disorders	Lupus erythematoses, Sjogren, Rheumatoid arthritis, Glomerulonephritis, Angiitis		
	Langerhan's histiocytosis		
Eye	Optic neuritis		
	Blunt and penetrating injuries, Infections, Sarcoid, Sickle C disease, Retinal detachment, Temporal arteritis		

<i>Cell, tissue or organ</i>	<i>Dysfunction or pathology</i>	<i>Condition or disease</i>	<i>Type</i>
	Retinal ischemia, Macular degeneration, Retinitis pigmentosa, Arteriosclerotic retinopathy, Hypertensive retinopathy, Retinal artery blockage, Retinal vein blockage, Hypotension, Diabetic retinopathy, and Macular edema		
Embryonic and fetal disorders	Asphyxia		
	Ischemia		
CNS	Chronic fatigue syndrome, acute and chronic hyposmolar and hyperosmolar syndromes, AIDS Dementia, Electrocutation		
	Encephalitis	Rabies, Herpes	
	Meningitis		
	Subdural hematoma		
	Nicotine addiction		
	Drug abuse and withdrawal	Cocaine, heroin, crack, marijuana, LSD, PCP, poly-drug abuse, ecstasy, opioids, sedative hypnotics, amphetamines, caffeine	
	Obsessive-compulsive disorders		
	Spinal stenosis, Transverse myelitis, <u>Guillian</u> <u>Guillain</u> Barre, Trauma, Nerve root compression, Tumoral compression, Heat stroke		
ENT	Tinnitus		
	Meuniere's syndrome		
	Hearing loss		
	Traumatic injury, barotraumas		
Kidney	Renal failure	Acute, chronic	Vascular/ischemic, interstitial disease, diabetic kidney disease, nephrotic syndromes, infections, injury, contrast-induced, chemotherapy-induced, CPB-induced, or preventive
	Henoch S. Purpura		
Striated muscle	Autoimmune disorders	Myasthenia gravis Dermatomyositis Polymyositis	

<i>Cell, tissue or organ</i>	<i>Dysfunction or pathology</i>	<i>Condition or disease</i>	<i>Type</i>
	Myopathies	Inherited metabolic, endocrine and toxic	
	Heat stroke		
	Crush injury		
	Rhabdomyolysis		
	Mitochondrial disease		
	Infection	Necrotizing fasciitis	
Sexual dysfunction	Central and peripheral (e.g. erectile dysfunction)	Impotence secondary to medication, (diabetes)	
Liver	Hepatitis	Viral, bacterial, parasitic	
	Ischemic disease		
	Cirrhosis, fatty liver		
	Infiltrative/metabolic diseases		
Gastrointestinal	Ischemic bowel disease		
	Inflammatory bowel disease		
	Necrotizing enterocolitis		
Organ transplantation	Treatment of donor and recipient		
Reproductive tract	Infertility	Vascular	
		Autoimmune Uterine abnormalities Implantation disorders	
Endocrine	Glandular hyper- and hypofunction		